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EXAMINER	
STRZELECKA, TERESA E	

ART UNIT	PAPER NUMBER
1637	

DATE MAILED: 05/23/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/936,095	STEMPLE ET AL.	
	Examiner	Art Unit	
	Teresa E. Strzelecka	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *10 March 2005*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-13* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-13* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *4/8/05*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. This office action is in response to an amendment filed March 10, 2005. Claims 1-23 were previously pending, with claims 14-23 withdrawn from consideration. Applicants amended claims 1 and 2 and cancelled claims 14-23. Claims 1-13 are pending and will be examined.

2. Applicants' amendments overcame the objection to claims 1 and 2. All previously made rejections are maintained for reasons given in the "Response to Arguments" section below.

3. Applicants' amendment to specification did not overcome the objection to specification regarding the first paragraph claim to priority. In the amendment to the first paragraph Applicants indicate that the instant application is a continuation of PCT/GB00/00873, which is incorrect, since the instant application is a national stage of PCT/GB00/00873.

Information Disclosure Statement

4. The information disclosure statement (IDS) submitted on April 8, 2005 was filed after the mailing date of the non-final office action on September 10, 2004. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Specification

5. The disclosure is objected to because of the following informalities: amendment to the specification containing the claim to priority is incorrect in that it lists the instant application as being a continuation of PCT/GB00/00873, whereas it is a national stage of this application.

Appropriate correction is required.

Response to Arguments

6. Applicant's arguments filed March 10, 2005 have been fully considered but they are not persuasive.

A) Applicants argue that the combination of Ross et al. and Williams would not lead to Applicants' claimed invention, because:

i) Ross et al. do not immobilize the polymerase on solid support,

ii) Ross et al. do not teach a single-molecule detection approach,

iii) Ross et al. use multiple nucleic acid templates, therefore their method is directed to multiple label molecules, whereas claim 1 teaches detection of a label incorporated into a single molecule of nucleic acid,

iv) Williams teaches a method of sequencing where a single molecule of nucleic acid is elongated on a solid surface,

v) Williams teaches detection of nucleotide addition using unblocked nucleotides, which have a fluorescent dye attached to the γ-phosphate, which is removed by the polymerase, therefore never incorporated into the elongating nucleic acid,

vi) "Like Ross, Williams does not teach the detection of a label which has incorporated into a single molecule of nucleic acid as in the claimed invention."

Regarding i), Ross et al. teach immobilization of polymerase on solid support via immobilized template-primer complex. Applicants do not claim direct immobilization of the polymerase onto the solid support. Williams teaches immobilization of polymerases directly on the solid support.

Regarding ii), Applicants do not claim a single-molecule detection approach, understood as detection of only a single nucleic molecule. Further, it is not clear what a "single molecule detection" would mean in the context of a plurality of polymerases immobilized on a solid support and addition of a nucleic acid sample to all of the polymerases, as claimed in steps a) and b) of claim 1. If it means that each of the polymerases interacts with a single nucleic acid molecule at a

time, than this condition is also fulfilled by Ross et al., since each of their nucleic acid molecules is immobilized separately on solid support (see, for example, page 32, lines 10-30), and interacts with a single polymerase molecule.

Regarding iii), Applicants do not claim detection of a label incorporated into a single nucleic acid molecule at a time (see also discussion above).

Regarding iv), Applicants did not provide a paragraph citing Williams teaching of a single elongated nucleic acid molecule on a substrate. What Williams does teach is sequencing nucleic acid by immobilizing a nucleic acid polymerase or the target nucleic acid in a single molecule configuration (col. 2, lines 16-24) and an array of immobilized polymerases on solid support (col. 3, lines 11-22). Therefore, Williams teaches detection of sequencing reactions each one of which uses a single nucleic acid template.

Regarding v), even though Williams teaches detection of a label attached to the γ-phosphate of a nucleotide, the mechanism of detection of nucleotide incorporation is the same as the one of Ross et al. and claimed by Applicants, namely, detection of a label cleaved off the nucleotide after it is incorporated into the growing complement of a template.

Finally, regarding vi), Williams does teach incorporation of labels into single nucleic acid molecules.

Therefore, one of skill in the art would have no trouble at all immobilizing polymerases directly on solid support and performing a method of Ross et al. using polymerases such immobilized, since both Ross et al. and Williams teach detection of sequencing reactions using labels cleaved off the nucleotides. Further, Williams teaches an embodiment in which the sequencing reaction is performed by sequential addition of a single type of nucleotide (col. 2, lines 47-49), which is exactly what Ross et al. are doing.

B) Applicants further argue that there would be no motivation to combine Ross et al. and Williams, because Ross et al. teach sequencing of multiple nucleic acid molecules and blocked dNTPs, whereas Williams uses continuous method of polymerase chain elongation, and cannot use blocked oligonucleotides.

All of these arguments were addressed in part A). It is pointed out that simulataneous detection of all four nucleotides is one of the embodiments presented by Williams, who also teaches sequential addition of nucleotides one type at a time (col. 2, lines 47-49). Therefore, since Williams provides motivation for attaching polymerases directly to solid support, there is ample motivation to combine the references.

C) Applicants argue that there is no reasonable expectation of success since the methods of Ross et al. and Williams are incompatible, since Wiliams teaches continuous sequencing and therefore it cannot function with blocked nucleotides, and Ross et al. teach retaining the labels, whereas Williams does not. Applicants further argue that the method of Ross et al. "detects DNA chain". Most of these arguments were addressed above. Further, Ross et al. teach an embodiment in which the label removed from the nucleotide is detected (page 14, lines 27-36; page 15, lines 1-5). Therefore, since both Ross et al. and Williams teach sequential incorporation of nucleotides and detection of labels cleaved off the nucleotides, the combination of these two methods has a high expectation of success.

The rejection is maintained.

Claim Interpretation

7. The term "immobilizing a polymerase on a solid support" is interpreted as binding the polymerase to a solid support either directly, for example, covalently, or indirectly, via binding of the polymerase to a nucleic acid attached to a solid support, for example. Applicants did not

provide a definition of "immobilized polymerase", and only the following description (specification, page 3, first two lines of the fourth paragraph): "... a plurality of polymerase molecules is immobilized on a solid support through a covalent or non-covalent interaction".

8. The order of steps in the method of claim 1 is given weight because of the phrase in the preamble "comprising the sequential steps of". For that reason there was no rejection made under 35 U.S.C. 102(b) over Ross et al., who do not teach immobilization of the polymerase prior to contacting the polymerase with a nucleic acid sample and primers.

9. Applicants did not define the term "reaction center", therefore it is interpreted as any space on a solid support.

Claim Rejections - 35 USC § 103

10. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

11. Claims 1-13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ross et al. (WO 91/06678) and Williams (U.S. Patent No. 6,255,083 B1).

A) Regarding claim 1, Ross et al. teach a method of nucleic acid sequencing, the method comprising:

(b) providing a nucleic acid sample for each of the plurality of said polymerases and a plurality of different oligonucleotide primers, wherein the nucleic acid sample hybridizes to an oligonucleotide primer (Ross et al. teach providing a nucleic acid template (= nucleic acid sample) and primers, where the nucleic acid hybridizes to an oligonucleotide primer (Fig. 1A and 1B; page 10, lines 32-35; page 11, lines 1-20).);

(a) immobilizing a plurality of polymerases on a solid support wherein each polymerase is immoblized in a reaction center of said solid support (Ross et al. teach that either the primer or the template are immobilized on solid support (Fig. 1A and 1B; page 10, lines 32-35; page 11, lines 1-20; page 32, lines 10-35; page 33, 34). Ross et al. teach addition of a polymerase to the immobilized template-primer complex, therefore they teach immobilizing the polymerase indirectly on a solid support (Fig. 1A and 1B; Fig. 2; page 12, lines 11-14 and 21-27). Ross et al. teach sequencing a plurality of nucleic acid molecules (page 7, lines 1-3; page 15, lines 13-16), therefore they teach a plurality of polymerases immobilized on a solid support via a plurality of nucleic acid templates and primers.);

(c) providing four different nucleotides, each nucleotide being differentially-labeled with a detachable labeling group and blocked at the 3' portion with a detachable blocking group, wherein the polymerase extends the primer hybridized to the nucleic acid sample with the differentially-labeled nucleotide that is complementary to the sample nucleic acid (Ross et al. teach providing four differently labeled and blocked dNTPs to the reaction zone with the templete, primer and polymerase (page 12, lines 15-18 and 29). Ross et al. teach a detachable 3'-blocking group (page 20, lines 25-34; page 21, 22; page 23, lines 1-25) and a detachable labeling group which is bound to the blocking group (page 14, lines 19-26; page 21, lines 4-8 and 28-31), or to the base of the nucleotide (page 27, lines 33-36; page 28, 29). The polymerase extends the primer with a labeled nucleotide complementary to the sample nucleic acid (page 12, lines 22-27).);

(d) removing nucleotides that have not been incorporated in the primer (Ross et al. teach removal of unreacted (= not incorporated) nucleotides (page 12, lines 29-34).);

(e) detecting the labeled nucleotide incorporated into the elongating primer, thereby identifying the complement of the labeled 3'-blocked nucleotide (Ross et al. teach identifying the

complement of the labeled 3'-blocked nucleotide by detecting the label attached to it (page 13, lines 1-13; page 26-28).);

(f) separating the 3' blocking group and the labeling group from the incorporated nucleotide (Ross et al. teach separating the blocking group and the labeling group from the incorporated nucleotide (page 13, lines 14-22; page 23, lines 28-35; page 24, 25; page 27, lines 33-36; page 28).);

(g) removing the separated 3' blocking group and the separated labeling group of step (f) (Ross et al. teach removing the separated 3' blocking group and the labeling group (page 13, lines 22-24).);

(h) confirming separation and removal of the 3' blocking group from the nucleotide incorporated in the primer (Ross et al. teach identifying the complement of the labeled 3'-blocked nucleotide by detecting the label attached to it (page 13, lines 1-13; page 14, lines 30-34; page 26-28), therefore, since it is the labeled group attached to the blocking group that is detected and it is removed before detection, Ross et al. inherently teach confirming separation and removal of the blocking group from the nucleotide incorporated into the primer.); and

(i) repeating steps (c) through (g) until either no new nucleotides are incorporated in step (c) or the 3' blocking group persists in not being separated and removed in steps (f) and (g), whereby the order in which the labeled nucleotide in step (d) are detected corresponds to the complement of the sequence of at least a portion of the nucleic acid sample (Ross et al. teach repeating the steps until the complementary chain has been completed, thereby providing the sequence of the nucleic acid sample (page 13, lines 30-35).).

Regarding claim 2, Ross et al. teach separation of the blocking group and the labeling group by photochemical activation (page 25, lines 4-12).

Regarding claim 3, Ross et al. teach separation of the blocking group and the labeling group by chemical reaction (page 24; page 25, lines 1-3; page 28, lines 19-35; page 38, lines 30-36; page 39, lines 1-9) or enzymatically (page 25, lines 14-25; page 39, lines 12-22).

Regarding claim 4, Ross et al. teach fluorescent labels (page 13, lines 4-8; page 21, lines 29-31; page 26, lines 17-26).

Regarding claim 5, Ross et al. teach attachment of the labeling group to the blocking group (page 14, lines 19-26; page 21, lines 4-8 and 28-31).

Regarding claims 6 and 8, Ross et al. teach a 2-ntrobenzyl group (page 21, line 26).

Regarding claim 7, Ross et al. teach attachment of the labeling group to the base of the nucleotide with a detachable linker (page 27, lines 33-36; page 28, lines 1-4 and 19-35).

Regarding claims 9 and 10, Ross et al. teach DNA polymerases, Taq (=DNA polymerase from Thermus acquaticus) and Klenow fragment of DNA polymerase I (page 19, lines 17, 18).

Regarding claims 9 and 12, Ross et al. teach an AMV reverse transcriptase (page 19, lines 1, 18).

B) Ross et al. do not teach a polymerase immobilized directly on a solid support at optically resolvable distance from each other, an RNA polymerase or detection of labeled nucleotides by total internal reflection fluorescence microscopy (TIFR), photon confocal microscopy, surface plasmon resonance and fluorescence resonance energy transfer (FRET).

B) Regarding claim 1, Williams teaches a method of nucleic acid sequencing, the method comprising:

(a) immobilizing a plurality of polymerases on solid support wherein each polymerase is immobilized in a reaction center of said solid support, and wherein said solid support comprises a plurality of reaction centers each located at an optically resolvable distance from each other

(Williams teaches immobilizing a plurality of nucleic acid polymerases onto a solid support in defined locations (= reaction centers), where the comparative information between sites can be optically recorded (col. 3, lines 11-17). Williams teaches immobilization of polymerases on solid support with very high spatial resolution (col. 4, lines 59-67; col. 5, lines 1-4; col. 14, lines 38-43), and detection of single molecules (col. 12, lines 23-39).)

(b) providing a nucleic acid sample and a plurality of different oligonucleotide primers, wherein the nucleic acid sample hybridizes to an oligonucleotide primer (Williams teaches providing a nucleic acid template and primers, where the nucleic acid hybridizes to an oligonucleotide primer (col. 2, lines 21-26).);

(c) providing four different nucleotides, each nucleotide being differentially-labeled with a detachable labeling group, wherein the polymerase extends the primer hybridized to the nucleic acid sample with the differentially-labeled nucleotide that is complementary to the sample nucleic acid (Williams teaches providing four differently labeled dNTPs and the polymerase extending the primer to create complement of the target nucleic acid (col. 2, lines 26-32).);

(e) detecting the labeled nucleotide incorporated into the elongating primer, thereby identifying the complement of the labeled 3'-blocked nucleotide (Williams teaches identifying the complement of the labeled nucleotide by detecting the label attached to it (col. 2, lines 32-35; col. 4, lines 4-22).).

Regarding claims 9 and 11, Williams teaches T7 RNA polymerase and E. coli RNA polymerase (col. 10, lines 65, 66).

Regarding claim 13, Williams teaches detection of fluorescently labeled pyrophosphates using TIFR (col. 12, lines 23-39 and 59-67).

It would have been *prima facie* obvious to one of ordinary skill in the art to have used the RNA polymerase of Williams in the method of Ross et al. The motivation to do so, provided by Williams, would have been that the T7 RNA polymerase and RNA polymerase from E. coli had a fidelity of at least 99% and a processivity of at least 20 nucleotides (col. 10, lines 59-62).

It would have been *prima facie* obvious to one of ordinary skill in the art to have used the TIFR detection method of Williams in the sequencing method of Ross et al. The motivation to do so, provided by Williams, would have been that TIFR detected single molecules with a signal-to-noise ratio of 12:1 at visible wavelengths (col. 12, lines 64-67).

It would have been *prima facie* obvious to have used an immobilized polymerase of Williams in the method of Ross et al. The motivation to do, provided by Williams, would have been that immobilization of polymerases allowed for analysis of single nucleic acid molecules which were obtained directly from an organism without the need for cloning or amplification and multiple nucleic acids were sequenced simultaneously (col. 1, lines 54-63), and, as stated by Williams (col. 13, lines 63-67): "Tethering of the polymerase, rather than the target nucleic acid (template) is convenient because it provides for a continuous sequencing process where one immobilized enzyme sequences many different DNA molecules.".

12. No claims are allowed.

Conclusion

13. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the

mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Teresa E. Strzelecka whose telephone number is (571) 272-0789. The examiner can normally be reached on M-F (8:30-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

May 17, 2005

JEFFREY FREDMAN
PRIMARY EXAMINER
5/17/05